Optimal Sample Preparation to Characterize Corrosion in Historical Photographs with Analytical TEM

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Abstract: An alternative focused ion beam preparation method is used for sampling historical photographs containing metallic nanoparticles in a polymer matrix. We use the preparation steps of classical ultra-microtomy with an alternative final sectioning with a focused ion beam. Transmission electron microscopy techniques show that the lamella has a uniform thickness, which is an important factor for analytical transmission electron microscopy. Furthermore, the method maintains the spatial distribution of nanoparticles in the soft matrix. The results are compared with traditional preparation techniques such as ultra-microtomy and classical focused ion beam milling.

Key words: sample preparation, focused ion beam, ultra-microtomy, conservation of cultural heritage, composite materials, nanoparticles

INTRODUCTION

Gelatin silver prints (and negatives) were the most used and widespread photographic materials in the 20th century. They can be found in large numbers in art collections, archives, family collections, and scientific documentation. The traditional black and white photographs are based upon the reaction of silver salts with light. Depending on the amount of exposure, the silver salts are converted to metallic silver. The unexposed silver salts are removed during fixation and the final image consists of metallic silver particles with a higher density of particles suspended in the gelatin for darker regions. Over time, the image particles react with oxidizing pollutants in the environment causing the particles to oxidize and silver ions to migrate through the emulsion. Depending on humidity the ions migrate further from the original image particles, which are dispersed in the gelatin matrix. At the surface of the emulsion the silver ions react with sulfuric pollutants and form clusters of small nanoparticles (Di Pietro & Ligterink, 2002). The crystal-line compounds at the surface influence the readability and esthetics of the image. The discoloration in Figure 1 is a typical result of silver degradation in historical glass negatives. Often, the discoloration is located around the edges and in areas with a high image density. To stop and possibly reverse the degradation, conservation scientists have investigated treatment methods such as atmospheric plasma to stabilize and potentially remove the degradation without altering the underlying image. In order to directly observe changes in the corrosion layer after treatment it is necessary to use a sample preparation method that does not influence characterization of the individual nanoparticles nor alter the spatial distribution of the corrosion in the soft matrix and on the surface of the negative. Moreover, in order to evaluate the degradation and conservation and restoration treatment, a high spatial resolution characterization technique such as transmission electron microscopy (TEM) should be used.

Preparing a sample is a crucial step in characterization of materials with TEM. When the samples are composite materials that contain both soft and hard matter, this step can be very challenging. Indeed, conventional techniques such as ion milling or ultra-microtomy often do not yield good results owing to large differences in response of the various components to the thinning technique. Focused ion beam (FIB) technology has recently become the method of choice for high-resolution work for a broad range of heterogeneous materials. In addition, in conservation science, FIB in combination with scanning electron microscopy (SEM) was recently introduced as a sample preparation method. It has been used to prepare samples of paintings (Casadio et al., 2011; Monico et al., 2011), ceramics (Lubelli et al., 2013) and wooden objects (Giachi et al., 2009). In this paper, we evaluate ultra-microtomy, FIB, and the combination of both as an approach for the TEM characterization of historical photographs consisting of a soft gelatin matrix and hard silver image and corrosion particles.

Procuring a sample from art or historical objects is not a straightforward process. As pointed out by the American Institute for Conservation in their code of ethics, the gain of sampling should be weighed against the effect of removing original material. Therefore, it is paramount that the amount of removed material is minimal. Fortunately, TEM is capable of providing detailed characterization from very little material. The conventional method for TEM sample preparation in the photography industry is ultra-microtomy with sections of ca. 100 nm thickness. A modern photograph on a polymer
can be directly cut with an ultra-microtome. However, in historical photographs the support is often glass or paper and the emulsion is more fragile so the sample has to be resin-embedded or sampled with FIB. The present report focuses on the different aspects of this TEM sample preparation, including some first results.

Materials and Methods

For the evaluation of plasma as a conservation method, historical glass negatives with visual corrosion were used. The selected examples are from the beginning of the 20th century and are made from Agfa Gevaert silver bromide negatives. They have been stored in their original cardboard box in a non-conditioned environment. As the objects show visual corrosion it can be concluded that the method of preservation was not optimal, as is the case with most historical objects.

In this work, we compare three sample preparation methods for images on a glass support: classical ultra-microtomy, standard FIB directly from the historical object, and an FIB sample made from the blockface of an ultra-microtome block. For classical ultra-microtomy the gelatin layer was first removed from the glass by chemically assisted mechanical pealing. Then a cut of 1 x 2 mm² of the layer was chemically fixed with a mixture of 4% formaldehyde and 2.5% glutaraldehyde in a cacodylate buffer of 0.1 M pH 6.9 for 24 h at 4°C. Then the sample was washed in the same buffer and dehydrated with a step gradient of ethanol at room temperature. This was then followed by stepwise infiltration of the gelatin with epoxy (Spurr resin) in a concentration ratio of 2:1 (100% ethanol:Spurr resin), 1:1 (100% ethanol:Spurr resin), 1:2 (100% ethanol:Spurr resin), and 100% Spurr resin. After embedding the sample in a flat mold, polymerization was performed at 60°C for 14 h, after which the blockface was trimmed with a histo diamond knife. A Reichert ultracut S ultra-microtome (C. Reichert AG, Vienna, Austria) was used to obtain semi-thin samples of around 100 nm, whereas sections of 100 nm to 30–40 nm thickness were produced with a Leica EM UC7 ultra-microtome (Leica, Vienna, Austria) using an ultra 45° diamond knife.

For standard FIB sample preparation a dual beam FIB/SEM (FEI Helios Nanolab TM650, FEI, Eindhoven, Netherlands) with a Ga⁺ source for thinning was used. The specimens were prepared in accordance with the wedge-cut method followed by an in situ lift-out technique (Bals et al., 2007). A carbon layer for electric conductivity and a protective Pt strip were applied to the emulsion. The Pt layer was applied in two stages, i.e., an electron beam-assisted deposition followed by an ion beam-assisted one. This sequence preserved the morphology of the corrosion layer. The lamella was then thinned using a voltage of 16 kV and a low current (0.4 nA). The use of low voltage (2–5 kV) and current (0.2–0.8 nA) during the final cleaning prevented preferential thinning between the soft matrix and the nanoparticles and removed any redeposition that might have occurred during the thinning steps. The final thickness was <100 nm.

In the third approach we used the blockface of the ultra-microtomed sample for preparation of the FIB lamella. This technique is based on serial sectioning of biological materials as suggested by Knott et al. (2008). The difference between classical ultra-microtomy and ultra-microtomy followed by FIB is the last stage or sectioning of the material. In Figure 2, a schematic overview of the different preparation steps is shown. After the classic prepreparation and embedding, a square block of the top with the blockface was cut and mounted onto an SEM stub. To increase stability under the electron beam a thin layer of amorphous carbon was evaporated onto the sample. The rest of the sectioning was similar to standard FIB preparation. In Figure 3, the different FIB steps show the selection and thinning of the region of interest (ROI). This region, highlighted by the Pt protective layer in Figure 3a, is perpendicular to the corrosion layer visible as a thin white line on the secondary electron SEM image. With this method the FIB lamella contains both the epoxy layer (left), the region of the nanoparticles or...
corrosion layer, and the infiltrated gelatin epoxy matrix containing image particles (right). In Figure 3b, the preserved corrosion layer can be seen as an interface between the different regions of the entire sample and in Figure 3c the final TEM lamella with the corrosion layer is shown.

Conventional TEM images and energy-dispersive X-ray spectra were acquired with an FEI G2 FEG Tecnai TEM (FEI, Eindhoven, Netherlands) operated at 200 kV. The same instrument together with a JEOL 3000F equipped with a Schottky FEG emitter gun operated at 300 kV was used for high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM), the latter also being used for energy-filtered transmission electron microscopy (EFTEM) maps using a Gatan imaging filter.

**RESULTS AND DISCUSSION**

**Ultra-Microtomy**

Ultra-microtomy is often used as a TEM preparation tool for soft materials. To cut the material into sections it needs to be chemically fixed and embedded in an epoxy. However, these steps can influence the original structure of the soft material (Grandfield & Engqvist, 2012). Fortunately, as the photographic gelatin was already partially hardened during production, no signatures of reduction of interfacial regions or sample heating and shrinkage were noticed in the present work. In addition, no changes to morphology of the particles were found after chemical fixation, as suggested by Kejser (1995). However, during the cutting of the sections, deformations such as compression, folding, chatter, and tears (Hayat, 1989) were introduced using both ultra-microtome instruments. These artifacts influence characterization of the spatial distribution and size of the particles in and at the corrosion layer. For example, Figure 4a shows folding of the section with the surface corrosion layer overlapping with internal image particles.

The amount of deformation can be determined by measuring the thickness and uniformity of the section. Large variations (up to 100%) in the intensity profile as shown in the inset of Figure 4b, and obtained from the marked area in the HAADF-STEM image, show that the section is not uniform in thickness or density over large micron-sized areas. The TEM image in Figure 4c is a rare case of a relatively uniform area as obtained with the Leica instrument. The thickness map shown in the inset was acquired with

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**Figure 3.** Combination of ultra-microtomy and FIB: (a) scanning electron microscopic image of the surface of a block-face of an ultra-microtome sample with the application of Pt protection layer over the region of interest; (b) focused ion beam wedge-cut of transmission electron microscopic lamella; (c) final FIB thinning of the lift-out sample clearly showing the corrosion layer.

**Figure 4.** Classical ultra-microtomy: (a) HAADF-STEM image with folding of a section (Reichert); (b) HAADF-STEM image showing variation in thickness in the gelatin (Reichert); (c) transmission electron microscopic image of intact corrosion layer with thickness map (Leica). HAADF-STEM, high-angle annular dark field scanning transmission electron microscopy.
EFTEM routine mapping in Digital Micrograph™ using the mean-free-path of the epoxy (Aronova et al., 2007) to compare the set thickness of the ultra-microtome with the actual sample thickness. Although in Figure 4c a corrosion layer is shown where the set thickness is close to the measured local value of 45 ± 10 nm, which is necessary for proper analytical work, the success rate of obtaining an intact interface is small with these thin sections.

**FIB**

The classic FIB sample was made directly from the historical photograph without any prepreparation such as chemical fixation or embedding. Objects of limited dimension can be mounted in the FIB/SEM so only a small amount of the original material was used. The corrosion layer is located just under the conductive carbon layer and protective Pt strip. Although there are several advantages to FIB/SEM preparation such as the selection of the location and the possibility of milling different materials while preserving the interface (Grandfield & Engqvist, 2012), artifacts can also be present, especially with soft materials (Edwards et al., 2009; Bassim et al., 2012; Schaffer et al., 2012). A disturbing feature is seen in Figure 5a, where low stiffness of the gelatin yields buckling during FIB thinning, resulting in extra thinning in the central area, even completely removing the Pt protection layer. In Figure 5b, some curtaining is seen: the intensity profile obtained from the marked rectangle does not contain any particles again showing that the sample is not uniform, which relates to the local changes in grayscale of the STEM image. Still, by reducing the milling voltage and current during the cleaning phase, the curtaining effect can be reduced. In another sample shown in Figure 5c, several holes are seen in the gelatin matrix. These holes can be the result of local beam induced heating owing to the low conductivity of the gelatin (±0.3 W/mK) (Fraga & Williams, 1985). Again, by lowering the current of the beam the local heating artifacts can be minimized (Kim et al., 2011). Any implantation of Ga⁺ during FIB does not disturb characterization of the corrosion layer as the Ga can easily be distinguished from the Ag and S peaks.

It can be concluded that TEM lamellae made by classical FIB can be used to study the particles, but the bending, thermal heating, and preferential milling still influence the spatial distribution and hamper local chemical characterization of the...
n nanoparticles. Experiments show that it remains difficult to make a uniform TEM lamella, which is thin enough for analytical characterization.

Ultra-Microtomy + FIB

By combining the preparation and blockface of the sample prepared by classical ultra-microtomy and FIB sectioning as shown in Figures 2 and 3 we can produce a TEM lamella with a clear interface that is thin enough to determine the chemical composition and distribution of the nanoparticles in the corrosion layer. At first, the added fixation, dehydration, and embedding steps of the emulsion layer strengthens the gelatin, thus reducing any artifacts in the final FIB lamella allowing thinner samples. The main advantage of the third method, however, is the change in orientation of the corrosion layer with respect to the Ga⁺ ion beam. The corrosion layer on the surface of the gelatin now forms an interface with the strengthening embedding epoxy (see Figs. 2, 3) and is also parallel to the incoming Ga⁺ beam. As a result, when using a slightly grazing incidence beam, one can easily produce a thin section containing the corrosion layer halfway in the sample, a region still being enforced by the surrounding epoxy matrix on both sides of the corrosion layer. In Figure 6a, an HAADF-STEM image shows the preservation of the corrosion layer.

In Figure 6a, an HAADF-STEM image shows preservation of the corrosion layer. The intensity profile in Figure 6b obtained from the square in Figure 6a shows that the sample is uniform in the epoxy when scanned perpendicular to the milling direction. The marked epoxy area of the thickness map in Figure 6c has a local thickness of $75 \pm 10$ nm. From the uniform intensity profile and thickness map it can be concluded that the combination method provides a more uniform sample compared with the classical FIB or ultra-microtomy preparations, whereas retaining the spatial arrangement of the corrosion layer. Although the success rate of this combined procedure is markedly better than that of the two alternatives, the main challenge remains making a thin enough sample to perform analytical characterization.

Table 1. Comparative Table of Different Criteria for TEM Sample Preparation and the Evaluation of Corrosion in Historic Photographs on a Glass Substrate.

<table>
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<th></th>
<th>UM</th>
<th>FIB</th>
<th>UM + FIB</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spatial distribution/large areas</td>
<td>+</td>
<td>+ / −</td>
<td>+</td>
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<tr>
<td>Chemical elements</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Corrosion layer</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uniformity</td>
<td>−</td>
<td>−</td>
<td>+</td>
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UM, ultra-microtomy; FIB, focused ion beam.

Characterization of the Degradation Layer

The results of a qualitative evaluation of the preparation methods are summarized in Table 1. The combination method yields the best characterization of the corrosion layer. The sample is uniform owing to preparation of the ultra-microtomy technique and the selectivity of FIB. The high success rate of the sampling makes it possible to study the differences between degradation, original materials, and conservation treatments by comparing the morphology, spatial distribution, location, and composition of the nanoparticles. In Figure 7a, an overview of the emulsion containing image particles and a corrosion layer is shown: the blue color observed in Figure 1 is only seen when such a corrosion layer is present on the surface of the photograph. This corrosion layer is an agglomeration of nanoparticles with a gradient into the gelatin, as seen in Figure 7b. In Figure 7c, an EFTEM map revealing Ag (green), S (red), and O (blue) shows that the top layer contains both metallic Ag particles as well as Ag₂S particles.

Figure 7. a: Cross-section of a photographic emulsion with blue coloration; (b) high-angle annular dark field scanning transmission electron microscopy of nanoparticles in and underneath the corrosion layer; (c) energy-filtered transmission electron microscopic map of corrosion layer. Green, Ag; red, S; and blue, O.
Conclusions

By comparing sample preparation methods we can conclude that the combination of a preprepared blockface with FIB thinning results in a uniform sample with an intact corrosion layer. In spite of the retained composition the classical ultra-microtome sections are often deformed by compression so that the corrosion layer cannot always be investigated. Classical FIB can cause preferential milling if the difference between the hard particles and soft matrix is too large. In contrast, it enables direct sampling from the art object and high selectivity of the ROI. The usefulness and success rate of the combination method are shown with HAADF-STEM and EFTEM mapping. This alternative FIB preparation method enabled us to prepare a high precision TEM lamella of the corrosion layer on a soft matrix. In addition, the method provided a thin section in which we could study the spatial, size and chemical distribution of the degradation layer as well as the individual nanoparticles. This combined preparation method can of course be used to also study other types of composite materials containing a hard surface layer on a soft substrate. In conservation science this can be a degradation phenomenon such as foxing on an old manuscript or the corrosion of silver thread in textile objects. In modern applications we encounter this type of material in, e.g., ink jet printing, organic light-emitting diodes, and flexible photo-voltaic materials.

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References


